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Bioavailability of microplastics to marine zooplankton: effect of shape and infochemicals

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ABSTRACT

The underlying mechanisms that influence microplastic ingestion in marine zooplankton remain poorly understood. Here we investigate how microplastics of a variety of shapes (bead, fibre and fragment), in combination with the algal-derived infochemicals dimethyl sulfide (DMS) and dimethylsulfoniopropionate (DMSP), affect the ingestion rate of microplastics in three species of zooplankton, the copepods *Calanus helgolandicus* and *Acartia tonsa,* and larvae of the European lobster *Homarus gammarus*. We show that shape affects microplastic bioavailability to different species of zooplankton, with each species ingesting significantly more of a certain shape: *C. helgolandicus* – fragments ($P<0.05$); *A. tonsa* – fibres ($P<0.01$); *H. gammarus* larvae – beads ($P<0.05$). Thus, different feeding strategies between species may affect shape selectivity. Our results also showed significantly increased ingestion rates by *C. helgolandicus* on all microplastics that were infused with DMS ($P<0.01$), and by *H. gammarus* larvae and *A. tonsa* on DMS-infused fibres and fragments ($P<0.05$). By using a range of more environmentally relevant microplastics, our findings highlight how the feeding strategies of different zooplankton species may influence their susceptibility to microplastic ingestion. Furthermore, our novel study suggests that species reliant on

chemosensory cues to locate their prey may be at an increased risk of ingesting aged microplastics in the marine environment.

INTRODUCTION

Microplastic (microscopic plastic, 1 µm-5 mm) is abundant and widespread in the marine environment and has been identified as a contaminant of global environmental and economic concern (1–3). The risks that larger plastic debris presents to marine organisms have been well documented (4–7), yet there still remain knowledge gaps regarding the impact of microplastics (1). Microplastics are not uniform, they are a complex array of different shapes, sizes and polymers (8). Microbeads and fibres can enter the environment via multiple pathways with direct release from waste water treatment works being a substantial source through the use of plastics in cosmetics and synthetic clothing (9,10). The degradation of larger plastic debris due to ultra-violet (UV) radiation and wave action can form irregularly shaped microplastic fragments (10,11). Due to their small size, microplastics are bioavailable via ingestion to a wide range of organisms (12). Ingestion of microplastics has been recorded in many marine species including cetaceans (13,14), seabirds (15), molluscs (16) and zooplankton (17,18). In species at lower trophic levels, such as zooplankton, ingested microplastics have been shown to cause several detrimental effects including reduced feeding behaviour, growth and fecundity (19–21).

Zooplankton are a crucial food source and provide an important link in the marine food web between phytoplankton and higher trophic levels (22). Zooplankton comprise of many different species of marine invertebrates and some vertebrates (e.g. fish larvae) including those species that spend their entire life cycle (holoplankton), and those with larval stages (meroplankton), in the plankton. Meroplanktonic species develop into adults that are often important constituents of fin-fish and shellfish stocks which are ecologically and economically important (23). Previous laboratory studies have shown that zooplankton have the capacity to ingest several different types of microplastics (17,24) which has also been documented in zooplankton from the wild (18,25,26). Whilst many studies have investigated microplastic presence and effect of ingestion, the underlying mechanisms and factors that influence plastic ingestion still remain poorly understood.

Food selectively has been widely evidenced in copepods, with the capacity to discriminate between algal prey and microplastics (27,28). There are several abiotic and biotic factors which can affect the biological availability (bioavailability) of microplastics to an organism (8,29). Whilst the overlap in size of the microplastic and the gape of the individual's mouth is key to ingestion and capture efficiency, other factors such as microplastic shape may affect handling and the capacity for ingestion (8,28). Many previous laboratory studies have used polystyrene microspheres that have been shown to be readily ingested by a number of species indicating that this shape is bioavailable to a broad range of taxa (17,19,30). However, several studies from the field investigating microplastic ingestion in zooplankton found that microfibres were most commonly ingested (18,25,26). It is unclear whether this

shape is more bioavailable or whether it is the most abundant microplastic in the areas sampled. Recent research has shown that different shaped microplastics can alter the severity of certain biological effects (31,32). For example in sheepshead minnow larvae (*Cyprinodon variegatus*), irregularly shaped microplastic fragments negatively affect swimming behaviour, decreasing the total distance travelled and their maximum velocity (32). Additionally Cole *et al*., (2019)(31) showed that the presence of fibrous microplastics can significantly alter prey selectivity in the copepod *Calanus finmarchicus*. These negative effects could reduce food intake and available energy for growth, development and reproductive success.

There is growing evidence that chemosensory cues, not just physical factors, could influence the bioavailability of microplastic (24,33–35). In the marine environment, plastic can provide a durable substrate for biofouling biota that may produce infochemicals (36,37). Colonization by infochemical-producing microorganisms, and subsequently the formation and growth of biofilms, could lead to plastic debris acquiring a chemical signal that is attractive to those species that use chemosensory mechanisms when locating, identifying and ingesting food (33,38–40). One such chemical is dimethyl sulfide (DMS), a marine trace gas derived from dimethylsulfoniopropionate (DMSP) that is produced by phytoplankton (39). DMS concentrations typically range from 1-7 nM in the surface ocean, with peak concentrations in the North Atlantic Ocean during June-July owing to the annual coccolithophore and dinoflagellate blooms (41). Recent research has identified that many species demonstrate foraging behaviour in the presence of DMS, including loggerhead turtles (42), seabirds (33), hard corals (34) and copepods (43). The chemical precursor DMSP has also been shown to induce swimming and aggregation behaviour in forage fish (44,45). Breckels *et al.* (2013) demonstrated that DMS plumes stimulated grazing behaviour response of *C. helgolandicus* at concentrations of 1.8 nM to 13.1 nM (46). Recent research by Procter *et al*. (2019) showed that *C. helgolandicus* ingested significantly more DMS-infused microfibres than virgin microfibres. This indicates that complex chemosensory cues may have a role in mediating foraging behaviour and therefore consumption of microplastic debris.

Currently little is known about what factors influence the uptake of microplastics by zooplankton. In order to better understand the mechanisms behind microplastic ingestion, it is vital to use microplastics that are more representative of those found in the marine environment (8,47). In the present study, we investigate the effect of microplastic shape and the presence of the infochemicals DMSP and DMS on microplastic ingestion by three species of zooplankton: the widely distributed suspension feeder *Calanus helgolandicus*, a temperate calanoid copepod and dominant mesozooplankton species in the North Atlantic; the globally distributed calanoid copepod *Acartia tonsa*, an ambush and suspension feeder; and the ambush feeder larvae of *Homarus gammarus* (European lobster) – a species of both economic and social importance in the UK. We test the hypotheses that: 1) species-specific ingestion is significantly different between microplastics of various shapes; and 2) infusion

of microplastics with DMS or DMSP significantly increases the bioavailability and ingestion of the differently shaped microplastics.

METHODS

Zooplankton sampling and husbandry

Zooplankton samples were collected from the Western Channel Observatory station L4, UK (50°15'N, 4°13'W) using 200 µm WP2 plankton nets in February 2019. Samples were transported within insulated boxes, containing natural seawater, to Plymouth Marine Laboratory (Plymouth, UK) within 3 hours of sampling. Adult female *C. helgolandicus* were identified using a dissecting microscope (Wild M5-49361; x20-x50 magnification) through assessment of their life stage, size, shape and distinct genital pore. Individuals were carefully picked out using Storkbill forceps and transferred to 5 litre beakers containing filtered seawater. Seawater was filtered via a filtration rig through a 0.22 µm nitrocellulose filter (Millipore, USA). European lobster larvae (*H. gammarus)* (stage 1) were obtained in August 2018 from The National Lobster Hatchery, Padstow, UK. Adult *A. tonsa* were provided from culture by Reefshotz, UK in September 2019. Female *A. tonsa* were identified using a dissecting microscope through assessment of their size, shape and distinct genital pore. All samples were processed and experiments conducted in controlled-temperature (CT) laboratories matched to the ambient sea surface temperature (SST) of 18 °C (*H. gammarus*-August 2018) and 10 °C (*C. helgolandicus*-February 2019) or culture temperature of 21 °C (*A. tonsa*).

Preparation of microplastics

Virgin 20 µm polystyrene beads (Spherotech, Illinois, USA), fresh cut virgin 20 µm x 10 µm nylon fibres (Goodfellow Cambridge Ltd., prepared following Cole (2016) method (48)) and virgin ≤20 µm nylon fragments (Goodfellow Cambridge Ltd.) were used to represent our three shape classifications (Supporting Information, Figure 1). All microplastics used were yellow in colour. The different polymers were used as at the time no nylon microbeads were available. Whilst the size of the microplastics was kept as uniform as possible, due to the different shapes, surface area (beads: 1.26 x 10^3 µm^2, fibres: 0.79 x10^3 µm^2, fragments: >1.26 x 10^3 µm^2) and volume (beads: 4.19 x 10^3 µm^3, fibres: 1.57 x 10^3 µm^3, fragments: <4.19 x 10^3 µm^3) varied.

For the experiments with *C. helgolandicus* and *A. tonsa*, glass DURAN experimental bottles (500 mL, total volume 615 mL) were ~75% filled with 0.22 µm filtered seawater (FSW) and spiked with the 15 mL vials of either beads, fibres or fragments of virgin (control) or DMSP- or DMS-infused beads, fibres or fragments. The bottles were then carefully filled to the brim with FSW which resulted in an overall concentration of 80 microplastics mL^{-1} in each of the experimental bottles. All microplastic treatments were incubated in MilliQ water in 15 mL gas-tight vials in a refrigerator at 3 °C for 3 days before use in grazing experiments.

Environmentally relevant concentrations of DMSP and DMS were chosen for our infused treatments (41,49). DMSP-infused beads/fibres/fragments were prepared by infusion in an aqueous 20 nM DMSP solution (Centrum voor Analyse, Spectroscopie and Synthese, Rijksuniversiteit Groningen, The Netherlands). DMS-infused beads/fibres/fragments were prepared by infusion in a 5 nM aqueous DMS solution (Sigma Aldrich Company Ltd.). The addition of infused microplastics to experimental flasks was performed by directly adding the entire 15 mL solution to the flask at the start of the experiment. This addition to the 600 mL of seawater in the flask resulted in a negligible calculated change in ambient DMSP/DMS concentrations of 0.49 nM (DMSP) and 0.12 nM (DMS).

The same process was used for the European lobster larvae. However, due to their cannibalistic nature under limited food availability, larvae were treated individually using smaller experimental bottles (50 mL) and gas-tight vials (1.9 mL). The concentrations of microplastics, DMSP and DMS remained the same.

At the microplastic concentrations used in this experiment, we were unable to measure final concentrations of DMS/DMSP fused to the microplastics as the levels are far below the detection limit of our methods.

Grazing experiments

For all species and for each shape of microplastic, the grazing experiments consisted of: (1) a virgin microplastic control group; (2) a DMSP-infused microplastic treatment group; (3) a DMS-infused microplastics treatment group. Following initial results with *H. gammarus* larvae, we refined the protocol in the *C. helgolandicus* and *A. tonsa* experiments to include additional controls of: (4) DMSP and microplastics added to experimental bottles separately but concurrently (DMSP non-infused); (5) and, DMS and microplastics added to experimental bottles separately but concurrently (DMS non-infused). Copepods and lobster larvae were starved for a period of 24 hours prior to the experiment.

In the *C. helgolandicus* and *A. tonsa* experiment, there were six replicates per treatment for virgin, infused/non-infused DMSP and DMS treatment groups, using copepods that had been acclimatized to the laboratory conditions for two days. The algae *Dunaliella tertiolecta*, *Prorocentrum micans* and *Thalassiosira rotula* were provided as a source of prey during the acclimation period for *C. helgolandicus*. They were cultured on f/2 media, with addition of silica for *T. rotula*, and maintained at 13 °C at a 16:8 light/dark cycle. *A. tonsa* copepods were maintained on their culture media of *Tetraselmis suecica*, *Isochrysis galbana* (T-Iso) and *Chaetoceros muelleri*.

Grazing experiments were carried out in gas tight 500 mL Pyrex bottles (total volume 615 mL), filled to the brim with 0.2 µm filtered seawater (FSW). Five healthy adult female *C. helgolandicus* or *A. tonsa* were transferred to each experimental bottle, followed by the addition of microplastics with or without DMSP or DMS. Copepods were not added to the T_0

(time zero, beginning of the experiment) experimental bottles. The experimental bottles were secured to a plankton wheel, rotated at 5 rpm and left for 6 hours in the dark in a CT room at 10 °C (*C. helgolandicus*) or 21 °C (*A. tonsa*). After 6 hours the experiment was stopped and the copepods were removed from each experimental bottle by gently passing the contents of the bottle through a 150 µm mesh into a beaker. The water was returned to the bottle and stored at 3 °C for microplastic enumeration using a FlowCam (Fluid Imaging Technologies Ltd.; see below). The mesh containing the copepods was examined under a dissection microscope and any mortality recorded. Copepods were then transferred into an Eppendorf tube containing 1 mL of 4% recycled formalin.

In the *H. gammarus* larvae experiment, there were ten replicates per treatment for virgin, DMSP and DMS treatment groups, using lobster larvae that had been acclimatized to the laboratory conditions for two days. Frozen plankton (TMC Gamma Blister Red Plankton), was provided as a source of prey during the acclimation period.

Grazing experiments were carried out in gas tight 50 mL Pyrex bottles (total volume 65 mL), filled to the brim with 0.2 µm filtered seawater (FSW). One healthy European lobster larvae was transferred to each experimental bottle, followed by the addition of microplastics with or without DMSP or DMS. Lobster larvae were not added to the T_0 experimental bottles. The experimental bottles were secured to a plankton wheel, rotated at 5 rpm and left for 3 hours in the dark in a CT room at 18 °C. We then followed the same process as above in the experiments, preserving the individuals in 1 mL of 4% recycled formalin.

A FlowCAM (VS-4 series) was used in auto-image mode to count the number of microplastic particles within a sample and determine plastic concentration. For the analysis, 40 mL of sample was pumped at 2 mL min^{-1} through the flow chamber fitted with a 100 µm x 2 mm flow cell and a 10 x objective lens which captured images of particles at 20 frames s^{-1}. FlowCAM uses the software programme VisualSpreadsheet (v 3.4), which can sort images by selected characteristics such as length. Any images that were not microplastic particles were deleted and the remaining images saved as a new list which would then be used to generate a count of particles mL^{-1}. The FlowCAM was used to determine the initial microplastic concentration (T_0) and the post experimental microplastic concentration (T_6).

Grazing rates were estimated by comparing changes in the abundance of microplastics over the experimental period for differently shaped microplastics with or without the addition of DMSP and DMS. Ingestion rates (particles $organism^{-1}$ $hour^{-1}$) were calculated using an adapted version of the Frost (1972) equation which accounted for the absence of prey growth during the incubations (35,50).

The grazing coefficient (*g*) was calculated from Equation 1:

$$g = 0 - \log\frac{T6}{T0} x \frac{1}{T} \qquad \text{Equation 1}$$

Where T_0 is the concentration of microplastics mL^{-1} at the beginning of the experiment, T_6 (T_3 for *H. gammarus* larvae experiment) is the concentration of microplastics mL^{-1} at end of experiment (after 3 or 6 hours) and T is time in hours (Supporting Information, Table 1). The clearance rates (*F*, mL organism^{-1} hour^{-1}) were calculated from Equation 2:

$$F = \frac{V\ x\ g}{n} \qquad \text{Equation 2}$$

Where V is the volume of experimental bottle (mL), *g* is the grazing coefficient calculated in Equation 1 and n is the number of zooplankton per bottle. The ingestion rate (*I*) is then calculated using Equation 3:

$$I = F\ x\ T_0 \qquad \text{Equation 3}$$

Statistical analysis

All data were analysed using Microsoft Excel (2016) and the statistical software R (version 3.4.1, R Development Core Team 2017). Data were tested for normality using a Shapiro-Wilk test and homogeneity of variance was visually inspected to satisfy parametric requisites. A one-way analysis of variance (one-way ANOVA) and Tukey's post hoc tests were used to compare ingestion rates from grazing experiments. The significance level was set at α=0.05.

RESULTS

Influence of shape on microplastic ingestion

The copepod *C. helgolandicus* demonstrated a significant variation in the ingestion rates of differently shaped microplastics (**Fig. 1a,** one way ANOVA ($F_{(2,15)}$=3.78, *P* = 0.047). A post hoc Tukey test showed that fragments were ingested significantly more than beads (*P* = 0.043). Fragments were ingested at a mean rate (± SE) of 500.3 (± 83.9) fragments copepod^{-1} hour^{-1}, in comparison to the mean number of beads, 144.5 (± 38.4) beads copepod^{-1} hour^{-1} and fibres 251.9 (± 134.0) fibres copepod^{-1} hour^{-1} (**Fig.1a**).

H. gammarus larvae also exhibited a significant variation in the ingestion rates of the different microplastic shapes (**Fig. 1b**, one-way ANOVA ($F_{(2,26)}$=4.36, *P* = 0.0233)). A post hoc Tukey test showed that the beads were ingested significantly more than fibres (*P* = 0.026) and substantially more than fragments (*P* = 0.074). Beads were ingested at a mean rate (± SE) of 1138.7 (±133.4) beads lobster^{-1} hour^{-1}, in comparison to the mean number of fibres, 402.3 (± 45.6) fibres lobster^{-1} hour^{-1} and fragments, 530.7 (± 281.7) fragments lobster^{-1} hour^{-1}(**Fig. 1b**).

The copepod *A. tonsa* demonstrated a significant variation in the ingestion rates of different shaped microplastics (**Fig. 1c,** one way ANOVA ($F_{(2,15)}$=6.6, *P* = 0.009). A post hoc Tukey test showed that fibres were ingested significantly more than beads (*P* = 0.008) and substantially more than fragments (*P* = 0.06). Fibres were ingested at a mean rate (± SE) of 587.5 (±

243.4) fibres copepod^{-1} hour^{-1}, in comparison to the mean number of beads, 204.1 (± 87.3) beads copepod^{-1} hour^{-1} and fragments 471.5 (± 196.4) fragments copepod^{-1} hour^{-1} (**Fig.1c**).

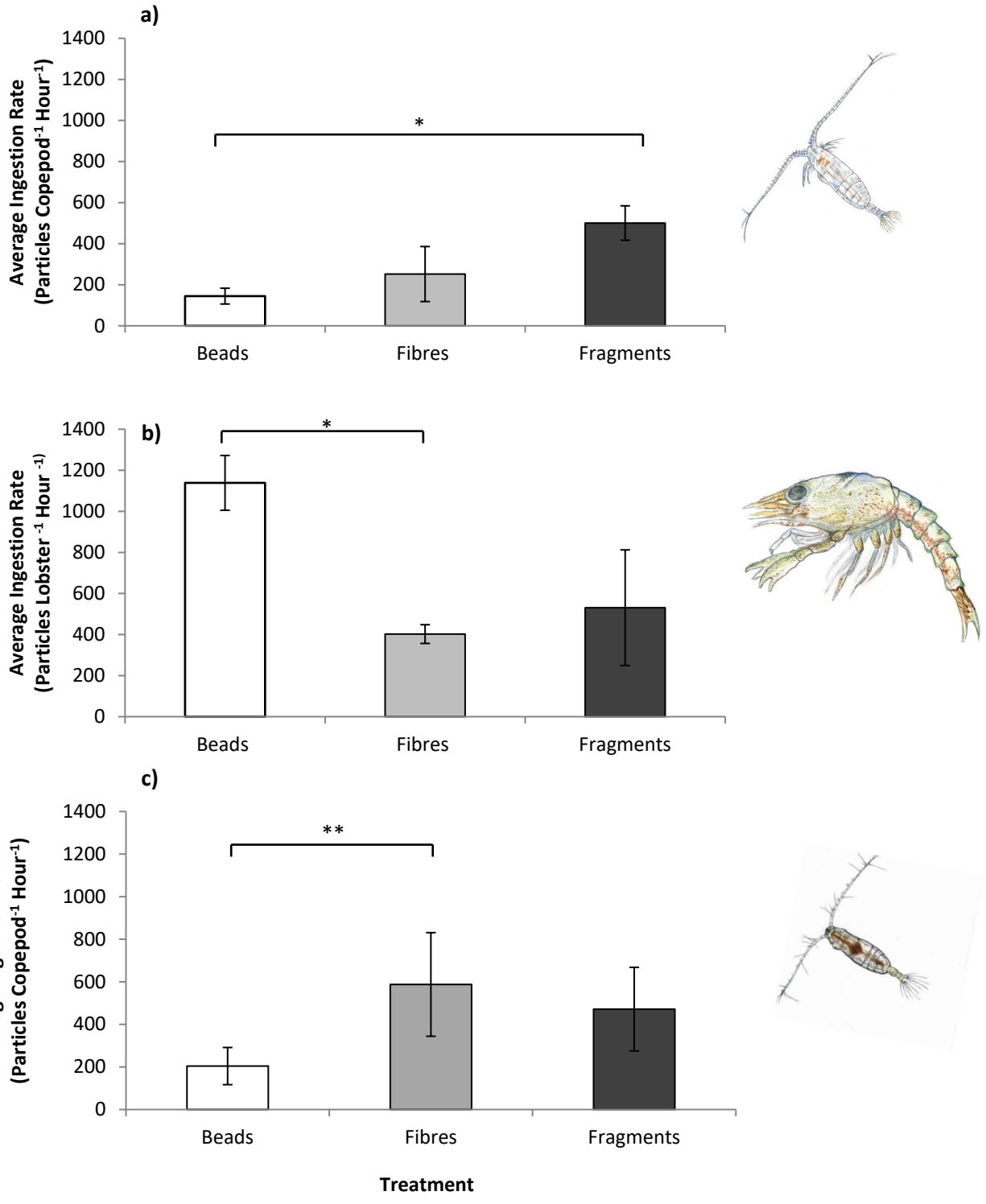

Figure 1. Ingestion rates ± SE (particles individual^{-1} hour^{-1}) of differently shaped microplastics including beads, fibres and fragments for a) *Calanus helgolandicus,* b) *Homarus gammarus* and c) *Acartia tonsa*. Asterisks denote level of significant difference in ingestion rates, * = P≤ 0.05, ** = P≤ 0.01. Illustrations by Vivienne Botterell.

Influence of infochemicals on microplastic ingestion

The copepod *C. helgolandicus* demonstrated an increase in the average ingestion rates of microplastics that had been infused with DMSP and DMS, across all three shapes, in comparison to virgin and also non-infused microplastics (infochemical and microplastics added to experimental bottles concurrently) (**Figure 2)**. Analysis showed that there were significant differences between the infochemical treatment groups with all three shapes (beads: **Figure 2a**, one-way ANOVA ($F_{(4,25)}$=6.235, P = 0.0013, fibres: **Figure 2b**, ($F_{(4,25)}$=8.214, $P \leq 0.001$) and fragments: **Figure 2c**, ($F_{(4,25)}$=15.21, $P \leq 0.001$)). A post hoc Tukey test showed that DMS infusion significantly increased the ingestion rates of beads **(Figure 2a**, $P \leq 0.001$), fibres (**Figure 2b**, $P \leq 0.01$) and fragments (**Figure 2c**, $P \leq 0.001$) in comparison to the virgin control treatments. In addition it showed that all microplastics that were infused with DMS were ingested significantly more than those that were not infused with DMS (beads: **Figure 2a**, $P \leq 0.05$, fibres: **Figure 2b**, $P \leq 0.001$, and fragments: **Figure 2c**, $P \leq 0.001$). This was also found for DMSP infused and non-infused fragments (**Figure 2c**, $P \leq 0.05$). There were no significant differences between the virgin control and the non-infused microplastic control for both infochemicals across all microplastic shapes.

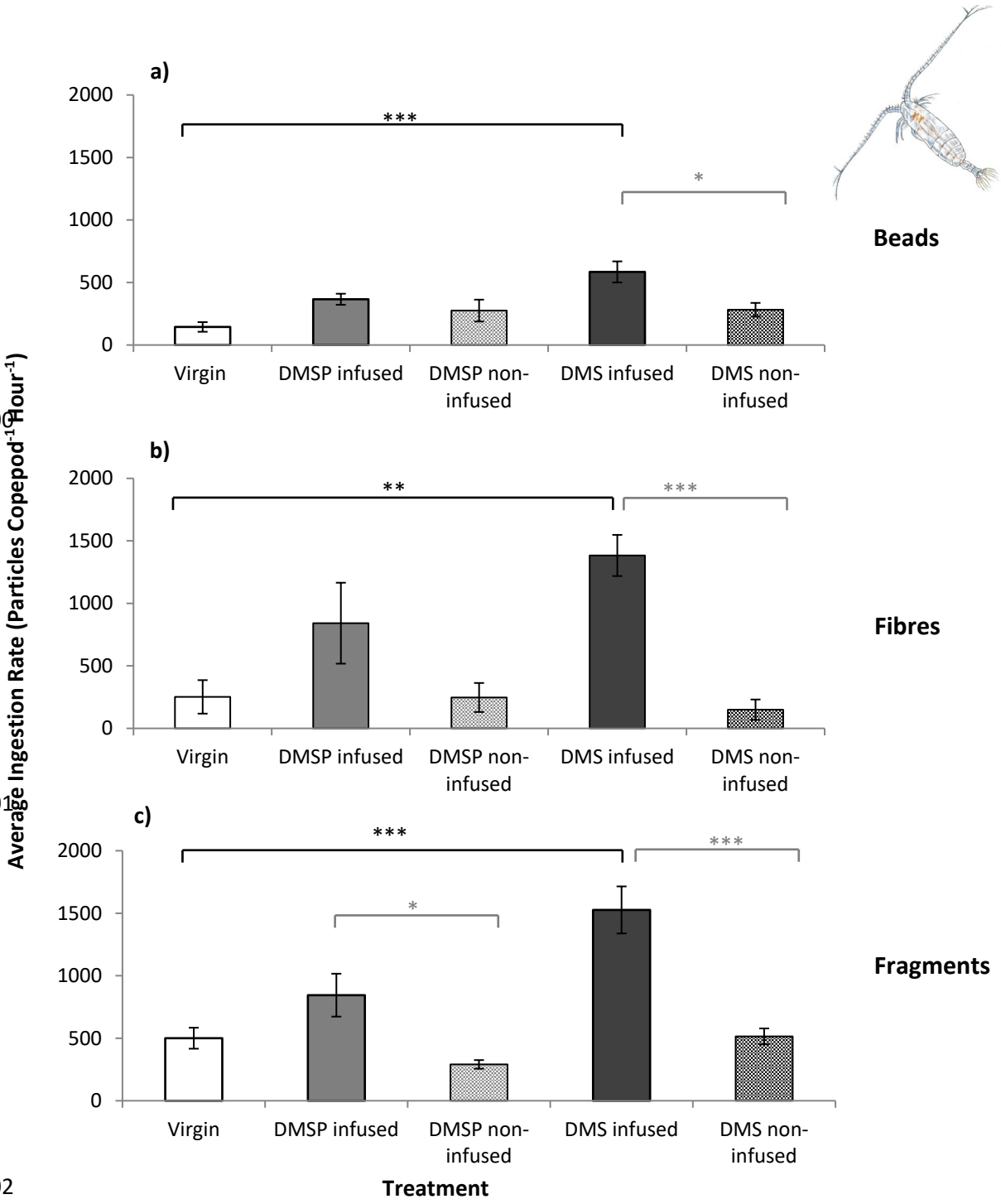

Figure 2. *Calanus helgolandicus* ingestion rates ± SE (particles copepod^{-1} hour^{-1}) of different shaped microplastics; a) beads, b) fibres and c) fragments, with the different treatments of virgin, DMSP infused, DMS infused, DMSP non-infused and DMS non-infused. Black significance bars relate to virgin controls, grey significance bars relate to non-infused controls. Asterisks denote levels of significant difference in ingestion rates, * = P≤ 0.05, ** = P≤ 0.01, *** = P≤ 0.001. Illustration by Vivienne Botterell.

In the DMSP and DMS infused treatments the average ingestion rate of fibres and fragments by *H. gammarus* larvae increased (**Figure 3**). Analysis showed that there were significant differences between the infochemical treatment groups for both fibres (**Figure 3b**, one-way ANOVA ($F_{(2,27)}$=4.481, P = 0.021) and fragments (**Figure 3c**, one-way ANOVA ($F_{(2,27)}$=6.372, P = 0.0054). A post hoc Tukey test showed that the presence of DMS significantly increased the ingestion rates of fibres (**Figure 3b**, $P \leq 0.05$) and fragments (**Figure 3c**, $P \leq 0.01$) in comparison to the control virgin treatments. The presence of the infochemicals had no effect on the ingestion rates of beads (**Figure 3a**, one-way ANOVA ($F_{(2,26)}$=2.863, P = 0.075).

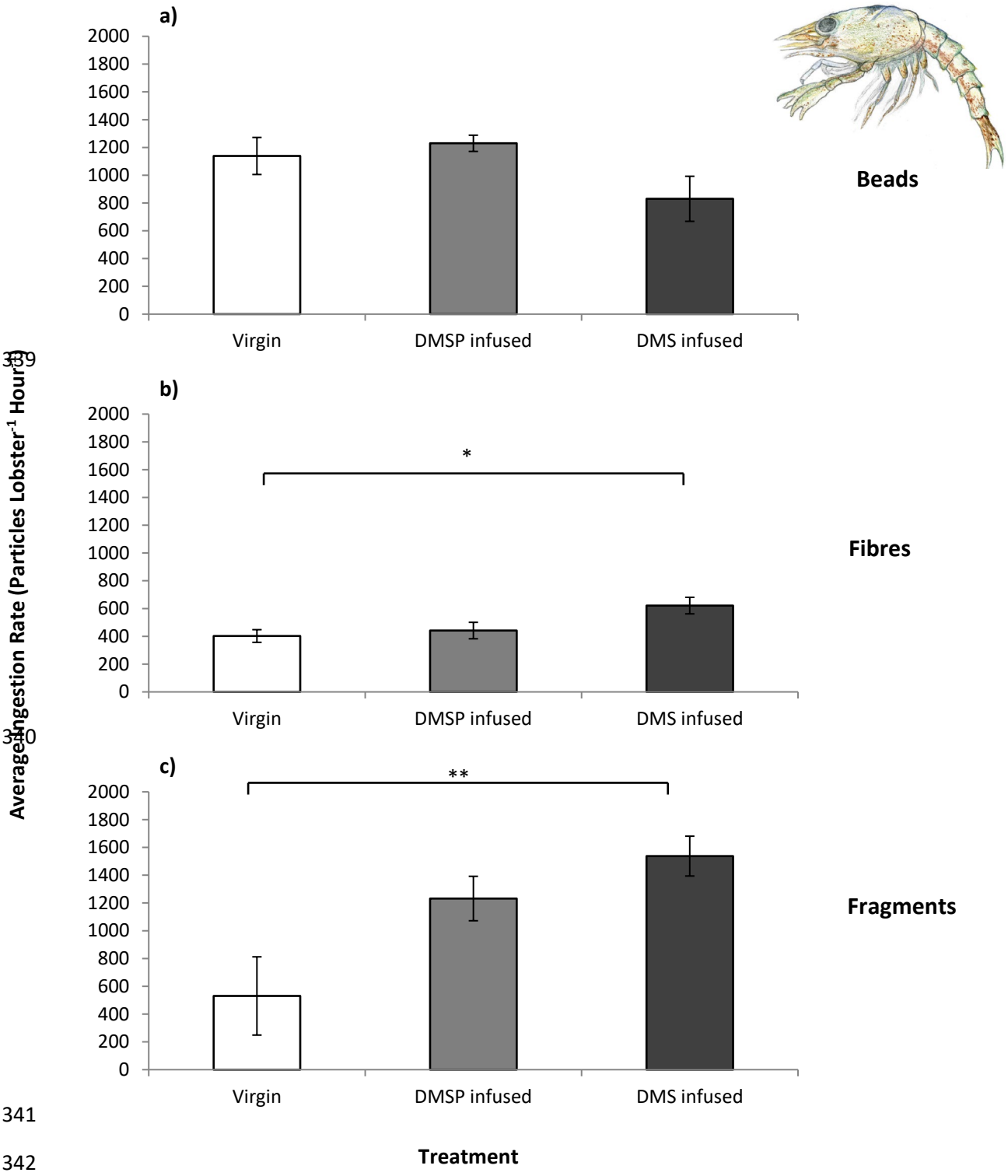

Figure 3. *Homarus gammarus* (larvae) ingestion rates ± SE (particles lobster^{-1} hour^{-1}) of virgin, DMSP infused and DMS infused microplastics for all three shapes: a) beads, b) fibres and c) fragments. Asterisks denote levels of significant difference in ingestion rates, * = P≤ 0.05, ** = P≤ 0.01. Illustration by Vivienne Botterell.

In the DMSP and DMS infused treatments the average ingestion rate of fibres and fragments by *A. tonsa* increased (**Figure 4**). Analysis showed that there were significant differences between the infochemical treatment groups for both fibres (**Figure 4b**, one-way ANOVA ($F_{(2,25)}$=11.6, $P \leq 0.001$) and fragments (**Figure 4c**, one-way ANOVA ($F_{(2,25)}$=21.2, $P \leq 0.001$). A post hoc Tukey test showed that the presence of DMS significantly increased the ingestion rates of fibres (**Figure 4b**, $P \leq 0.05$) and fragments (**Figure 4c**, $P \leq 0.01$) in comparison to the control virgin treatments. The presence of the infochemicals had no effect on the ingestion rates of beads (**Figure 4a**, one-way ANOVA ($F_{(2,25)}$=1.7, $P = 0.18$). In addition it showed that fibres and fragments that were infused with DMS were ingested significantly more than those that were not infused with DMS (**Figure 4b**, $P \leq 0.001$, and fragments: **Figure 4c**, $P \leq 0.001$). This was also found for DMSP infused and non-infused fragments (**Figure 4c**, $P \leq 0.01$). There were no significant differences between the virgin control and the non-infused microplastic control for both infochemicals across all microplastic shapes.

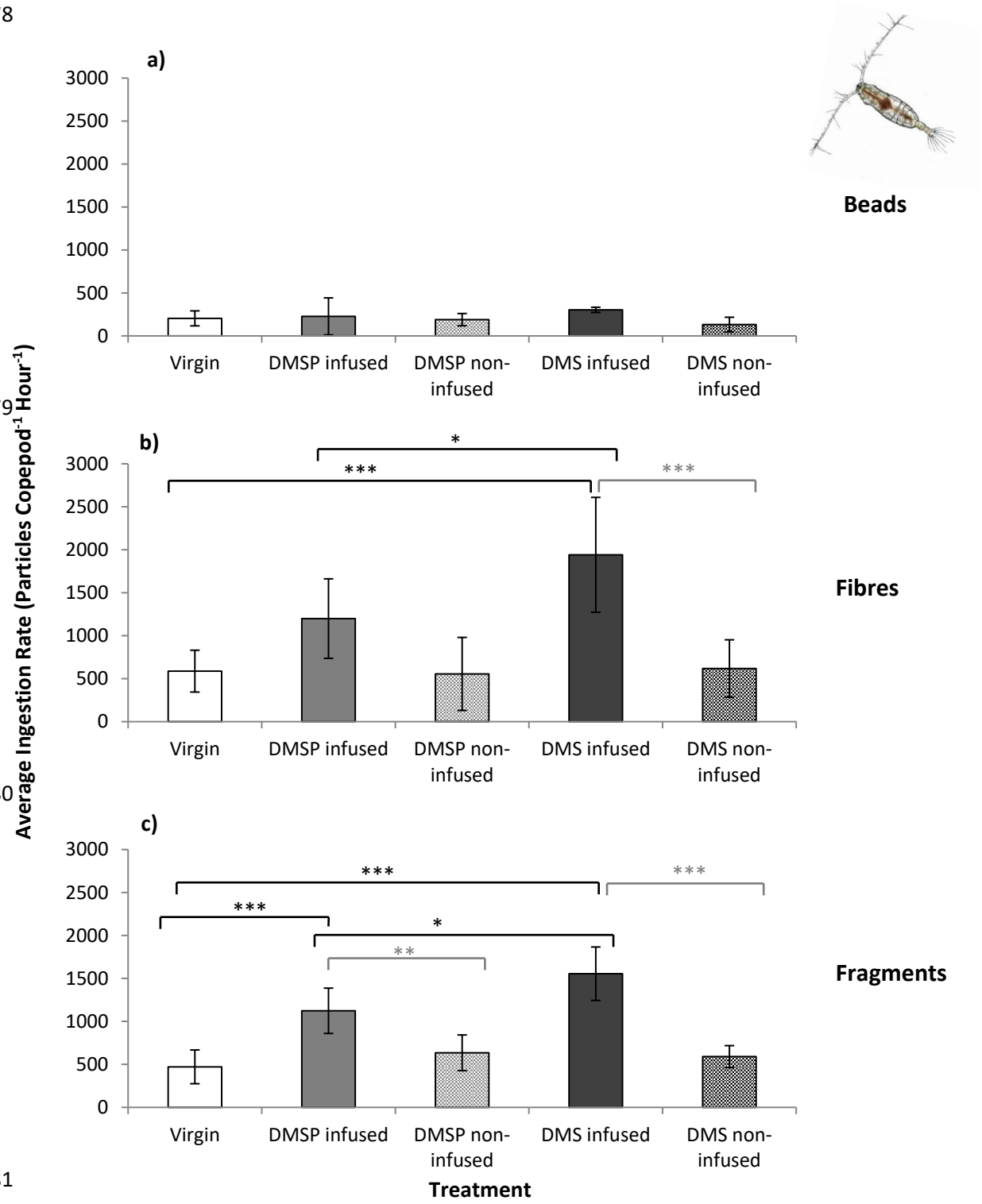

Figure 4. *Acartia tonsa* ingestion rates ± SE (particles copepod^{-1} hour^{-1}) of different shaped microplastics; a) beads, b) fibres and c) fragments, with the different treatments of virgin, DMSP infused, DMS infused, DMSP non-infused and DMS non-infused. Black significance bars relate to virgin controls, grey significance bars relate to non-infused controls. Asterisks denote levels of significant difference in ingestion rates, * = P≤ 0.05, ** = P≤ 0.01, *** = P≤ 0.001. Illustration by Vivienne Botterell.

DISCUSSION

Our study reveals that both shape and the infusion of infochemicals can affect the ingestion rate of microplastics in *Calanus helgolandicus*, *Acartia tonsa*, and *Homarus gammarus* larvae. Each species selectively ingested significantly more of a certain microplastic shape (**Fig. 1)**, indicating that shape is an important factor that influences microplastic bioavailability. *C. helgolandicus* ingested more fragments; *H. gammarus* ingested more beads and *A. tonsa* ingested the most fibres. We further observed that infusion with the infochemical DMS significantly increased ingestion rates of microplastics in all three species (**Fig. 2-4**). This highlights that chemosensory species utilising DMS as an infochemical may be at an increased risk of microplastic debris ingestion. These findings add to growing evidence of the importance of testing environmentally relevant microplastics in zooplankton grazing studies in contrast to the predominately used virgin beads, to fully elucidate the mechanisms behind microplastic ingestion (8).

Effect of microplastic shape on its bioavailability to zooplankton

Previous laboratory research has shown that many species of zooplankton will readily ingest microplastic beads including *C. helgolandicus* and *A. tonsa* (17,51). However microplastic ingestion has not previously been observed in *H. gammarus* larvae. Fragments and fibres have also been shown to be ingested by *C. helgolandicus* (28,31). Furthermore, in wild copepods sampled in the natural environment, irregularly shaped and fibrous microplastics have been identified (18,25). However it is unclear whether these microplastics from wild samples are due to prey selectivity of the species, differences in gut retention time or simply a representation of the most prevalent microplastic in the environment under investigation.

Our results show that whilst all microplastic shapes were ingested, each of the species selectively ingested one shape preferentially over the others (**Fig. 1**). The selectivity of the species could be explained by different feeding strategies, with particular shapes being easier to handle, or species-specific capacities to ingest. *C. helgolandicus* ingested the most fragments, *H. gammarus* the most beads and *A. tonsa* the most fibres. *C. helgolandicus* is a suspension feeder, using appendages around their mouths to generate a feeding current (52,53). Whereas *A. tonsa* is an ambush feeder, a complex grazing behaviour requiring a stimulus to optimise capturing prey items yet avoiding non-food items, but can also switch to suspension feeding when consuming small phytoplankton by generating a feeding current (54,55). It is the smallest of the three species used in these experiments and therefore may have found the smaller diameter of the fibres (10 µm) easier to ingest. Additionally it is possible that different shaped microplastics may generate different eddies, through disturbances in the feeding current or water flow, which may be more or less attractive to species with different feeding strategies. Similarly to *A. tonsa*, *H. gammarus* larvae are also thought to be ambush feeders consuming both phytoplankton and zooplankton (56). Unlike copepods which have a singular naupliar eye that is light sensitive, lobster larvae have two compound eyes which are not only light sensitive but have a large view angle and are able

to detect fast movement (57,58). This more developed spatial vision may aid the lobster in prey selection and subsequently may have played a role in the selectivity of microplastic beads over the other shapes. The microplastic shape may also resemble the species natural prey source. The microplastics used in this current study overlapped in size with the species prey. Beads and fragments may resemble spherical algae and fibres could resemble chain forming diatoms (28). Shape selectivity could also be explained by species shifting their prey preference (31). Recent research by Cole *et al*., (2019) and Coppock *et al*., (2019) suggest that *Calanus* species may shift prey selectivity to avoid ingesting microalgae that are a similar size and shape to microplastics that they were exposed to, potentially to avoid consuming the plastic particles (28,31). However future behavioural experiments are recommended to further understand this mechanism of microplastic shape selectivity.

Certain microplastic shapes have been shown to have more adverse effects than others in species of zooplankton, with previous research highlighting negative effects on swimming behaviour by fragments (32) and feeding behaviour by fibres (31). It is crucial to understand which shapes have the highest bioavailability in a species to understand the effect commonly ingested microplastic shapes could have on the health of the individual as any negative effects could reduce food intake and available energy for growth, development and reproductive success. Future experiments should consider differences in gut retention time between differently shaped microplastics as certain shapes may be retained longer than others. This is imperative to understanding whether the microplastics we find in zooplankton sampled in the natural environment were due to selectivity of the species, representative of microplastics present in the environment or are retained for longer in the gut. The length of time microplastics are retained for has implications not only for the health of the individual but also for the transport of microplastics through the water column by diel vertical migration of zooplankton and also through the food web when zooplankton is consumed by predators (28,59).

The role of infochemicals in increasing bioavailability of microplastics

To investigate whether these chemicals would stimulate grazing, *C. helgolandicus*, *A. tonsa* and *H.* gammarus larvae were exposed to microplastics (beads, fibres and fragments) that had been infused in artificial DMS and DMSP solutions of environmentally relevant concentrations. Our results show that the presence of the infochemical DMS can lead to significant increases in the ingestion rate of microplastics in three species of zooplankton. This indicates that chemosensory species utilising DMS as an infochemical may be at an increased risk of microplastic debris ingestion.

In *C. helgolandicus,* the ingestion rates of all three microplastic shapes were significantly increased when infused with DMS and whilst not significant, DMSP infusion also substantially increased microplastics uptake. *H. gammarus* larvae exhibited a similar pattern to *A. tonsa*, with significantly more microplastic fibres and fragments ingested when infused with DMS. However DMSP infusion only substantially increased microplastic fragment

ingestion in *H. gammarus* and neither infochemical affected the ingestion rate of microplastic beads. However DMSP infusion did significantly increase the ingestion rate of both fibres and fragments in *A. tonsa*, but like DMS had no effect on bead ingestion. Whilst the majority of the microplastics infused with DMS were ingested at a higher rate in all species, DMS infusion had no effect on the ingestion of microplastic beads by the lobster larvae. However microplastic beads in the virgin treatment group were ingested at the highest rate in comparison to fibres and fragments, which implies that the microplastic shape had a higher bioavailability and an overriding effect on the chemoattractive potential of DMS. It is possible that the more developed vison of the larvae likely aided in prey detection and selection, and that the larvae used a range of chemo- and mechanoreceptors in combination with visual cues. This highlights that in some species microplastic shape, based on particles that are easier to handle or mimic preferred prey items, may present a greater bioavailability than other factors including attraction by infochemicals.

Following initial results with *H. gammarus* larvae, we refined the protocol in the *C. helgolandicus* and *A. tonsa* experiments, to include additional controls in order to investigate whether simply the presence of infochemicals in the surrounding seawater induced an increase in ingestion rates. Here, the addition of virgin, non-infused microplastics to the experimental flasks was performed separately but concurrently to the addition of 15 mL DMS or DMSP solution (see Methods, Preparation of Microplastics). However, there was no significant difference in ingestion rates between the virgin controls and the non-infused controls across all microplastic shapes. Furthermore our results (**Fig. 2 & 4**) show that there was a significant difference between the non-infused DMS controls and the infused DMS treatments for almost all three different shapes of microplastics. This therefore suggests that it is not just the presence of DMS in the seawater, but the presence of DMS on the plastic itself which stimulates increased ingestion.

In the marine environment, the infochemicals DMSP and DMS could be present on the plastics surface due to biofilm formation by DMSP/DMS producing microorganisms which form part of the diverse microbial community of the plastisphere (38). Many of these organisms within the plastisphere are important prey items readily consumed by several species of zooplankton (60). This could increase the ability of zooplankton to locate the plastic particles if they mimic the scent of natural prey and in turn could increase consumption of microplastics. It is important to note that these experiments are a simplification of the natural environment separating visual biofilm effects from chemical cues associated with DMSP/DMS. There still remains a significant knowledge gap assessing the ability of microplastics to gain an infochemical signature through the formation of a biofilm. This work seeks to further understand the interspecies response to DMSP/DMS yet future investigation is still required to understand the response to additional infochemicals and importantly the detection thresholds of chemosensory organisms.

Environmental relevance and risk assessment

In this study we demonstrate that both shape and the presence of infochemicals can affect the ingestion rate of microplastics in three species of zooplankton. This research highlights the importance of using a greater diversity of environmentally relevant microplastics in laboratory experiments. For our infochemical infusion experiments we used environmentally relevant concentrations of DMSP and DMS. Whilst this triggered increased uptake of microplastics in all three of the species, currently there is very little understanding of the interspecies response to other infochemicals, limited knowledge on the detection threshold of chemosensory species, and a poor understanding of chemical gradients emanating from microscopic particles. The microplastics used in this study were chosen to be as similar as possible (i.e. size and colour), however due to the use of different shapes they did vary slightly in volume and surface area which could affect ingestion rates in some species. It's possible that those microplastics with a greater surface area, such as fragments, may gain a greater infochemical signature. Similarly polymer type could also affect ingestion rates. Different polymers have been shown to develop different microbial biofilm communities which in turn could produce different infochemicals (61). Whilst we used nylon fibres and fragments we also used polystyrene beads as we were unable to find a nylon equivalent. The concentration of microplastics used in this study exceeds those currently observed in the marine environment, however there is very little environmental information relating to microplastics in the size range of 10-30 µm due to technical difficulties in sampling, extracting and identifying such particles (31). However where data is available, they suggest an inverse relationship between particle size and abundance, hence, the smaller the microplastics the larger their concentration (62). Recent research by Lindeque *et al.*, 2020 has demonstrated that the 333 µm nets commonly used for microplastics sampling underestimate microplastic abundance, particularly for <333 µm microplastics that are within the optimal prey size range of numerous marine organisms (63). Whilst it is important to investigate the risk that environmentally relevant microplastic concentrations pose to marine organisms, it is essential to understand the mechanisms by which microplastics become bioavailable to a species, can cause harm, identify end points, understand the sensitivity of different species at every life stage and it is also crucial to conduct these studies at elevated/future scenario concentrations (8,31). Such research is key to establishing no-effect thresholds for the development of effective risk assessments for species, populations and the ecosystem.

SUPPORTING INFORMATION

This material is available free of charge via the internet at http://pubs.acs.org.

Images of the microplastics and microplastic concentration data at T_0, T_3 and T_6.

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